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# Neuroendocrinology of insulin resistance: metabolic and endocrine aspects of adiposity

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**Abstract**

Abdominal obesity is a major risk factor to attract the insulin resistance syndrome. It is proposed that abdominal obesity exposes the liver to elevated levels of free fatty acids, which activate a neuroendocrine reflex, leading to increased circulating levels of glucocorticoids. Besides directly attenuating peripheral insulin signaling, glucocorticoids oppose the activity of central nervous regulatory systems that stimulate insulin action. Among the factors that promote insulin action is leptin. Leptin regulates peripheral fuel partitioning and insulin action mainly through hypothalamic neuronal networks, which in turn, regulate endocrine activity of adipose tissue in a way comparable to thiazolidinediones. These are a class of insulin-sensitizing drugs, which exert their antidiabetic effects through the gamma isoform of peroxisome proliferator-activated receptor (PPAR-γ). Since glucocorticoids oppose leptin action at several levels of control (including the central nervous system, CNS), it is argued that subjects easily develop obesity and associated metabolic disorders.


*Keywords:* Fatty acid; Glucocorticoid; Leptin; Adiponectin; PPARγ (peroxisome proliferator-activated receptor); Hypothalamus

## 1. Introduction

Many species, including rodents and primates, eat their food in bouts (i.e., meals) that are interspaced by time intervals of different durations. This strategy is considered quite advantageous, since it allows animals to display a variety of behaviors of different intensities during the inter-meal interval. Some of these may be crucial for survival, and could have ultimately influenced the evolutionary success of some species. One implication of food eaten in short bouts is that the absorbed amount of fuels during and after a meal should greatly exceed direct metabolic requirements (Strubbe and van Dijk, 2002). To this end, the 'milieu interieur' is put at risk because food intake-associated fuel excursions can have deleterious effects on metabolic (e.g., glycosylation causing loss of enzymatic efficacy) and cardiovascular (e.g., triglyceride and cholesterol accumulation, atherosclerosis, high blood pressure, etc.) systems (Preus, 1997; Sowers and Epstein, 1995). In order to dampen fuel excursions in the general circulation associated with food intake, evolution has provided most higher species with an intricate system, which effectively stores absorbed nutrients into reserve tissues (Woods et al., 1984). The pancreas, and in particular one of its endocrine factors, insulin, plays a key role in this process. Among several nutrients (including several carbohydrates, fatty-, and amino acids), of which the levels are elevated in the circulation during and following a meal, a rise in the ambient circulating glucose level is a particularly strong stimulus to secrete insulin into the blood stream. Increased insulin secretion can already occur when food enters the pharyngeal cavity, and thus before fuels actually rise in the circulation (Strubbe and Steffens, 1975). This so-called "cephalic phase" of insulin secretion, together with the

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nutrient-stimulated insulin secretion, contributes to the optimised storage of energy fuels in the form of glycogen in liver and muscle, and of triglycerides in adipose tissue (Woods et al., 1984).

During inter-meal intervals, stored fuels can be released again and deliver energy required for metabolic purposes, such as tissue maintenance, heat production, muscle movement, etc. Lowering of insulin secretion is a major contributor to the release of glucose from the liver, thereby supplying energy to the central nervous system (CNS) and peripheral tissues. In particular, the CNS needs a constant supply of glucose, since glucose is a requisite fuel for the brain (Woods et al., 1984). In addition, a gradual decline in insulin secretion stimulates free fatty acid release from adipose tissue, and free fatty acids can be utilized efficiently by peripheral organs and tissues through the β-oxidation pathway (Strubbe and Prins, 1986). The fall in insulin secretion also contributes to the release of regulatory hormones which stimulate hepatic gluconeogenesis. This latter mechanism is important in the face of continued starvation, since hepatic glycogen stores are depleted after 1 or 2 days. Adipose tissue, on the other hand, is able to meet ongoing energy demands for several weeks, and thus becomes the main source of energy in the event of continued starvation (Cahill et al., 1966).

Given the important roles of insulin in the regulation of fuel fluxes in the body, it can be imagined that failure of the pancreas to secrete insulin, for example due to autoimmune destruction of the pancreatic B-cells (i.e., type-I Diabetes Mellitus), is perhaps one of the most drastic metabolic diseases known to date. As there is no cure for type-I Diabetes Mellitus, patients suffering from this disease are left with insulin replacement therapy. Another form of this disease is characterized by severe tissue resistance to insulin. At an early stage of this disease, absorbed nutrients associated with food intake require much more insulin to be stored into the reserve tissues. At a later stage, this has been proposed to underlie loss of B-cell functioning and concomitant hyperglycemia (Lingohr et al., 2002), a condition that is referred to as type II Diabetes Mellitus. Whereas type-II Diabetes Mellitus is traditionally regarded as a disease of the elderly, the incidence of this disease in young adults and even children is currently increasing (Rosenbloom et al., 1999). Up to 50–80% of subjects worldwide with Diabetes Mellitus are obese depending upon ethnic background and community. Some studies have shown that the occurrence of type II diabetes among obese individuals is more than 5–10 times higher than in normal weight individuals (Zimmet et al., 2001). Besides being often hyperphagic and obese, insulin-resistant subjects are frequently dyslipidemic, hypertensive (or have other cardiovascular complications), and have disturbances in neuroendocrine axis (Bjorntorp, 1992). The present review focuses on some of the potential mechanism that may relate these factors to insulin resistance.

## 2. Role of fat deposition in peripheral insulin action

### 2.1. Free fatty acids

The increased prevalence of the insulin resistance syndrome and obesity has paralleled the prosperity of our western-industrialized society, and therefore might be considered lifestyle-induced diseases. The most frequently mentioned factors that propel them are an increase in dietary fat intake in combination with a sedentary existence. Among the three macronutrients, i.e., fat, carbohydrate, and protein, fat indeed has the lowest satiety index and the highest caloric density (Flatt, 1986; Rolls et al., 1994), and thus would cause the largest weight gain in the form of stored body fat. Increased body fat, particularly the abdominal type, is often associated with increased lipolysis and elevated plasma levels of free fatty acids (Jensen et al., 1989). At present, it is becoming increasingly clear that chronic exposure to elevated plasma levels of free fatty acids can lead to elevated triglyceride content inside a number of organs and tissues other than adipose tissue (Boden and Shulman, 2002). In turn, intracellular free fatty acids in these non-adipose organs can interfere with the cellular mechanisms of insulin signaling and down-regulate cellular sensitivity to insulin (Shullman, 2002). This idea is corroborated by cross-sectional studies in humans using $^{1}H$ magnetic resonance spectroscopy where insulin resistance correlated strongly with the intramyocellular lipid concentration (e.g., Perseghin et al., 1999). Dobbins et al. (2001) observed that the interaction between increased free fatty acid exposure in rats caused by high-fat feeding and the development of insulin resistance is stronger when muscular β-oxidation of fatty acids is reduced. Thus, increased triglyceride content in muscle due to increased loading as well as reduced breakdown might be considered high-risk factors for the development of insulin resistance (Boden, 1997).

Besides interfering with muscular tissue sensitivity to insulin, free fatty acids are also known to affect the liver. It has for example been shown that experimental elevation of the plasma free fatty acid level increases hepatic glucose production and this is particularly due to a rise in gluconeogenesis (Roden et al., 2000). This may contribute to hyperglycemia and increased insulin production in type-II diabetic patients. Increased cholesterol synthesis has been observed under conditions of elevated plasma free fatty acid levels as well (Adeli et al., 2001). Since abdominal fat drains on the hepatic portal circulation, it seems reasonable to believe that increased abdominal fat deposition might particularly expose the liver to elevated free fatty acid levels, which in turn can causes hepatic insulin resistance (Bjorntorp, 1992). Consistent with this idea is the finding that surgical removal of visceral fat reverses hepatic insulin resistance in moderately obese rats (Barzilai et al., 1999). Benthem et al. (2000) exploited this idea further, and investigated whether

experimental elevation of the free fatty acid level in the hepatic portal vein of rats could directly induce insulin resistance in these animals. To this end, rats were chronically infused during a 24-h interval with oleic acid into the portal vein, and thereafter subjected to an intravenous glucose tolerance test. Consistent with the hypothesis was the finding that the plasma insulin concentration rose far higher in response to the intravenous glucose tolerance test relative to the control group whereas glucose levels were equally increased, which is a strong indication of reduced insulin sensitivity (see Fig. 1). The inhibitory effects on insulin sensitivity were not observed upon equimolar infusion of the medium-chain free fatty acid, caprylate, into the hepatic portal vein, indicating that the liver is more sensitive to long-chain fatty acids. The infused oleate may have increased hepatic triglyceride stores, which is presumably a committed step towards hepatic insulin resistance. This idea is consistent with the recent reports on the role of stearoyl-CoA desaturase (SCD) in catalyzing the biosynthesis of mono-unsaturated fatty acid enabling triglyceride synthesis. When fed a high-fat diet, mice with a targeted disruption of the $SCD_1$ isoform had dramatically reduced hepatic triglyceride storage as well as increased sensitivity to insulin and an increased rate of lipid oxidation (Ntambi et al., 2002). In addition, $SCD_1$ disruption in mice was shown to protect markedly against genetic obesity and concomitant insulin resistance (Cohen et al., 2002).

### 2.2. Glucocorticoids

Besides causing insulin resistance, feeding of a high-fat diet is often associated with increases in neuroendocrine activity, including that of the hypothalamo-pituitary adrenal axis (Tannenbaum et al., 1997). Provided that high-fat feeding increases the hepatic portal level of free fatty acids, this observation is consistent with findings in the study of Benthem et al. (2000) that hepatic portal oleic acid infusion causes increases in the circulating levels of the sympathetic transmitter, norepinephrine, and of corticosterone (see Fig. 1B). These latter effects were not observed following hepatic portal administration of equimolar amounts of caprylate, the medium-chain fatty acid. It is well known that increased circulating levels of glucocorticoids induce insulin resistance in peripheral tissues (Kusunoki et al., 1995; Olefsky et al., 1975; Rizza et al., 1982), and increased sympathetic activity might predispose to hypertension and related cardiac diseases (Tuck, 1992; Grekin et al., 1997). Thus, the findings in the study of Benthem et al. (2000) reinforce the idea originally proposed by Bjorntorp (1992) that increased levels of hepatic portal free fatty acids induce a reflex activation involving the neuroendocrine system, and this mechanism might contribute to the develoment of insulin resistance and related metabolic and cardiovascular derangements. Directly relevant to this idea is the finding by Orbach and Andrews (1973) that vagal afferent activity is increased following long-chain fatty acid infusion into the

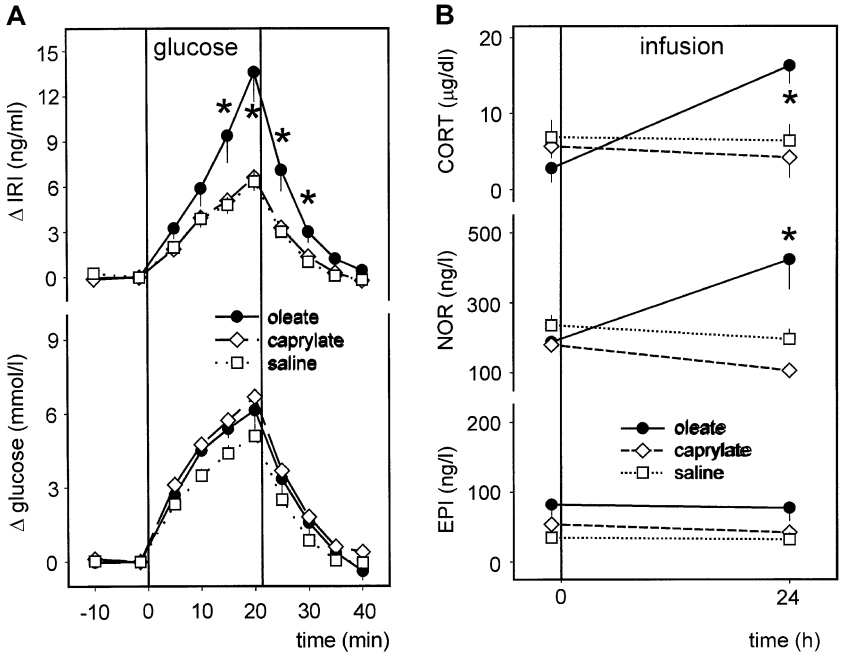


Fig. 1. (A) Plasma immunoreactive insulin (IRI) and blood glucose profiles in response to the intravenous glucose tolerance tests (16 mg/20 min) during intraportal fatty acid infusions (150 nmol/min) over the 24-h preceding interval in male Wistar rats. (B) Effect of 24-h intraportal fatty acid infusion (150 nmol/min) on plasma corticosterone, norepinephrine, and epinephrine levels in male Wistar rats. All data are expressed as average change ( ± S.E.M.). Asterisk indicates a significant different relative to saline treatment.

hepatic artery of rabbits. At present, we can only speculate about the mechanism by which a potential signal related to excess triglycerides and/or reduced lipid oxidation is able to activate vagal and/or visceral afferents and ultimately reaches the CNS. First of all, evidence from animal studies suggests that the activity of hepatic vagal/visceral afferent nerves can be modulated by metabolic processes within the liver (Lutz et al., 1996; Friedman, 1988). Another possibility might be that elevated levels of hepatic portal free fatty acids produce local inflammatory processes in the liver, and these could activate vagal/visceral afferents as well (Volpes et al., 1992). Consistent with this latter possibility is the findings in the study of Benthem et al. (2000) that hepatic portal oleic acid infusion caused an increase in plasma levels of alanine aminotransferase, a marker previously found to be associated with symptoms of syndrome X as well as hepatic steatosis (Hollmann et al., 1997).

A potential signal conveyed within hepatic vagal afferents could reach the brain stem, and in turn, could be connected to the paraventricular hypothalamus, a brain region known to regulate sympathetic outflow and activity of the hypothalamo-pituitary adrenal axis (Sawchenko and Swanson, 1981, 1985). Consistent with a role for vagal afferent nerves in insulin sensitivity and neuroendocrine activity are the findings of Koopmans et al. (1998), who showed that destruction of unmyelinated afferent nerves (including those from the liver) by the neural toxin capsaicin in rats greatly improves glycemic control in these animals, as well as markedly reduces the activity of the hypothalamo-pituitary adrenal axis and the sympathetic nervous system. Improved glycemic control by afferent neural destruction could be achieved as well in dramatically obese and type-II diabetic Zucker rats (Gram, 2003). Along other lines, Yuan et al. (2001) found that Zucker rats chronically treated with high doses of sodium salicylate augmented insulin sensitivity and improved glycemic control as well. Thus, while high doses of salicylates might improve insulin sensitivity through inhibition of the nuclear factor kappa B and its upstream activator IκB kinase β in peripheral tissues, these doses of salicylates certainly block cyclooxygenases, which are key enzymes involved in prostaglandin synthesis. The latter pathway is the classical target of nonsteroidal anti-inflammatory drugs such as aspirin. Since prostaglandins are produced in the CNS in response to activation of a vagal afferent pathway involving interleukins and corticotropin-relasing hormone (Ericsson et al., 1997), blockade of this pathway may result in reduced activation of the hypothalamo-pituitary-adrenal axis and the sympathetic nervous system, and therefore would ameliorate the metabolic syndrome. While Yuan et al. (2001) have not directly assessed the possibility that aspirin treatment affects neuroendocrine parameters in Zucker rats, this mechanism might be plausible considering the fact that inhibitors of cyclooxygenases have previously been shown to reduce the activity of the hypothalamo-pituitary-adrenal axis (Parsadaniantz et al., 2000). Important in this respect is the notion that Zucker rats, as well as many other genetically obese rodents, have an amplified activity of the hypothalamo-pituitary-adrenal axis (Walker et al., 1992). Taken together, high plasma levels of free fatty acids can underlie the insulin resistance syndrome either directly in peripheral tissue or via a stimulatory effect on activity of the hypothalamo-pituitary-adrenal axis resulting in elevated levels of glucocorticoids.

It needs to be added that increased action of glucocorticoids leading to the insulin resistance syndrome can be achieved even in the absence of increased circulating levels of glucocorticoids. For example, cases have been reported of obese humans who have normal glucocorticoid levels, but with increased activity of 11-β-hydroxysteroid dehydrogenase type 1 (11β-HSD) in fat tissue. 11 β-HSD plays a crucial role in determining the intracellular glucocorticoid concentrations by regenerating the active glucocorticoid (cortisol in humans and corticosterone in rodents) from inactive forms (Rask et al., 2001). Transgenic mice overexpressing 11β-HSD in fat tissue consistently become insulin-resistant, hyperlipidemic, and, surprisingly, also display hyperphagia despite increased body adiposity (Masuzaki et al., 2001). The mechanisms that control these processes are currently under intense investigation, and might involve a role for free fatty acids as well (Stulnig et al., 2002).

### 2.3. Leptin

In addition to factors that promote insulin resistance and related metabolic diseases, there are also mechanisms known to counterbalance these effects. Among these is leptin, a 167-amino-acid product of the OB gene predominantly expressed in adipose tissue (Zhang et al., 1994). It has been described above that defects in fuel partitioning into adipose tissue, either because of increased adipose tissue mass or increased lipolysis and circulating free fatty acids, results in dyslipidemia, hyperglucocorticoidism, insulin resistance and in some cases diabetes. Starting with the discovery of leptin in 1994 by Zhang et al. (1994), however, this ‘‘passive’’ view on adipose tissue is rapidly changing towards the concept that adipose tissue is an active endocrine organ involved in the regulation of energy metabolism and fuel partitioning (Mora and Pessin, 2002). Leptin circulates in proportion to body adiposity in many species including primates and rodents (Considine et al., 1996; Zhang et al., 1994). Among the tissues that express mRNA encoding the signaling form of leptin receptors are, for example, adipose tissue, muscle, liver, gastrointestinal tract, adrenal gland, and a number of regions in the CNS, including the ventrobasal hypothalamus (Schwartz et al., 2000). The role played by leptin in the regulation of energy balance is well addressed by the fact that the hyperphagia, insulin resistance, and obesity of Zucker rats as well as db/db mice (Ahima and Flier, 2000; Seeley et al., 1996) are due to mutations in the signaling variant of leptin receptors [i.e., *db/db* mice or *fa/fa* Zucker rats]. Mice with a mutation in the

ob gene [i.e., *ob/ob* mice (Ahima and Flier, 2000; Campfield et al., 1995)] yielding a dysfunctional form of secreted leptin are extremely obese, insulin-resistant and hyperphagic as well. Apparently, these animals lack a negative feedback signal from their fat stores, which would be necessary to allow appropriate adjustments to disturbances in adipose tissue mass. Recombinant functional leptin administration reverses obesity and metabolic derangements only in rodents that synthesize a deficient form of leptin, but not in those that have a dysfunctional leptin receptor (Campfield et al., 1995).

It is becoming increasingly clear that leptin has powerful insulin-sensitizing capacities. For example, loss of adipose tissue mass (lipodystrophy) in rodents as well as in humans, which causes a state of hypoleptinemia, results in a severe insulin resistance, dyslipidemia, hepatic fat deposition and potentially diabetes (Ebihara et al., 2001). Leptin replacement (Ebihara et al., 2001) as well as transplantation (Gavrilova et al., 2000) of non-mutant fat are able to reverse some of these abnormalities in lipodystrophic mice, and essentially the same data are currently collected in lipodystrophic humans receiving leptin therapy (Farooqi, 2003). With the transplantation studies, however, one needs to caution the fact that transplanted fat depots are not innervated, as opposed to endogenous fat (Fliers et al., 2002; Kreier et al., 2002), which may account for some differences in outcomes among studies. Nevertheless, the insulin resistance syndrome and dyslipidemia observed in lipodystrophic mice are homologous to those observed in leptin-deficient *ob/ob* mice, in spite of the huge differences in fat deposition among these strains. This indicates that it is not the difference in fat accumulation per se underlying these metabolic syndromes, but the absence of leptin.

### 2.4. Other factors related to adiposity

Following the discovery of leptin, a number of other genes and their products were found in adipose tissue with potent effects on fuel partitioning and metabolism. Among these is adiponectin (also known as AdipoQ or ACRP30), a highly conserved gene of which the expression as well its secreted peptide product into the circulating was found to be down-regulated in leptin-deficient *ob/ob* mice (Hu et al., 1996). Injection of purified adiponectin in mice stimulates β-oxidation in peripheral tissue and promotes fat depletion (Yamauchi et al., 2001), body weight loss (Fruebis et al., 2001), and causes a transient decline in basal glucose levels of *ob/ob* mice as well as in non-obese diabetic or streptozotocin-induced diabetic mice (Berg et al., 2001; Yamauchi et al., 2001). Adiponectin appears to be down-regulated in obesity, particularly when the latter is associated with the metabolic syndrome (Arita et al., 1999). The finding that leptin-deficient *ob/ob* mice have reduced plasma levels of adiponectin could imply that leptin up-regulates adiponectin synthesis and, vice versa, that leptin resistance in obese subjects underlies the reduction in adiponectin synthesis. Consistent with a role for leptin in adiponectin synthesis is the finding by Zhang et al. (2002) that peripheral leptin treatment prevented a fall in adiponectin synthesis in adipose tissue of rats, despite the fact that these rats were anorexigenic and had a profound loss in body weight. In rats pair-fed to these leptin-treated animals, a reduction in adiponectin synthesis was observed, presumably as a result of reduced leptin levels. Besides adiponectin and leptin, adipose tissue also produces factors which oppose insulin action. Among these, tumor necrosis factor α (TNF-α) might be the major candidate as it is up-regulated in obesity (Uysal et al., 1998), and impairs insulin action (Hotasmisligil, 1999). Another adipocyte hormone with insulin-desensitizing actions is resistin, but presumed up-regulation in obesity is highly debated (Steppan and Lazar, 2002; Way et al., 2001).

In this respect, it is interesting to note that adiponectin synthesis is down-regulated by glucocorticoids (Fasshauer et al., 2002), and up-regulated by thiazolidinediones (Combs et al., 2002; Maeda et al., 2001). Thiazolidinediones are a class of insulin-sensitizing drugs, which exert their antidiabetic effects through a mechanism that involves activation of the gamma isoform of peroxisome proliferator-activated receptor (PPAR-γ), a nuclear receptor (Spiegelman, 1998). Thiazolidinedione-induced activation of PPAR-γ alters the transcription of several genes involved in glucose and lipid metabolism and energy balance, including those that code for lipoprotein lipase, fatty acid transporter protein, adipocyte fatty acid binding protein, fatty acyl-CoA synthase, malic enzyme, glucokinase and the glucose transporter $GLUT_4$ (Hauner, 2002). High levels of TNF-α as a result of obesity and/or atherosclerosis suppress adiponectin synthesis and this effect can also be prevented by thiazolidinediones (Maeda et al., 2001). Through these mechanisms, thiazolidinediones reduce insulin resistance in adipose tissue, muscle and liver, and are used therapeutically in type-II diabetic patients. However, PPARγ is predominantly expressed in adipose tissue (Vidal-Puig et al., 1996). Thus, it is possible that the effect of thiazolidinediones to increase insulin action and normalize associated metabolic dysregulations in tissues occurs through stimulated adiponectin synthesis and release and/or changes in the release of other adipose hormones/factors.

## 3. CNS control of peripheral fuel metabolism and insulin action

While some of the effects of leptin on peripheral insulin action and metabolism may be attributed to autocrine effects of leptin on metabolic processes in peripheral tissues (Siegrist-Kaiser et al., 1997), or to the fact that leptin reduces glucocorticoid secretion at the level of the adrenal gland (Pralong et al., 1998), a major hypothesis states that leptin and glucocorticoids regulate energy metabolism and fuel partitioning via interactions with CNS neuronal networks.

### 3.1. Glucocorticoids

The spectrum by which glucocorticoids can affect energy balance is not only restricted to insulin sensitivity, but in fact, may permit most aspects of the metabolic syndrome in many genetically obese rodents. For example, removal of the adrenal glands in Zucker rats causes profound amelioration of insulin resistance (Freedman et al., 1986), but also leads to normalization of their plasma lipid levels and induces leanness (Castonguay et al., 1986). In addition, Zucker rats reduce their food intake, in particular that of dietary fat, upon adrenalectomy (Castonguay et al., 1986). The latter effect suggests a role of glucocorticoids mediated via the CNS. An interaction between glucocorticoids and CNS neuronal networks involved in feeding behavior has been substantiated by Leibowitz (1990). In this event, the diurnal rise in glucocorticoids which normally occurs preceding the active phase probably underlies an increase in the synthesis in arcuate hypothalamic neurons of neuropeptide Y (NPY), i.e., a neuropeptide with remarkably potent stimulatory effects of food intake (Leibowitz, 1990). Consistent with an anabolic action of glucocorticoids in the CNS are the findings of Zakrzewska et al. (1999a) that prolonged infusion of dexamethasone (i.e., a synthetic glucocorticoid agonist) over days into the cerebral ventricle of genetically normal rats induces obesity, hyperphagia and hypertriglyceridemia in these animals, despite their hyperleptinemia. These effects are probably mediated via NPY, since chronic glucocorticoid treatment in these animals was associated with increased synthesis of NPY in the arcuate hypothalamus. Increased levels of NPY in the paraventricular hypothalamus, which is an important projection area of arcuate hypothalamic neurons, induce obesity, hyperphagia, hypertriglyceridemia and insulin resistance, but again, these effects rely on central glucocorticoid action (Zakrzewska et al., 1999b). In addition, increased NPY levels in the paraventricular hypothalamus cause a shift in autonomic outflow to a higher parasympathetic activity, which, for example, leads to elevated insulin responses during meals, and this contributes to subsequent weight gain and increased adipose tissue mass as well (Van Dijk et al., 1994). Since paraventricular NPY is known to stimulate glucocorticoid secretion (Albers et al., 1990), interactions among free fatty acids, glucocorticoids, and paraventricular NPY can easily become self-perpetuating, and could therefore propel towards development of the metabolic syndrome, particularly when subjects are fed a high-fat diet.

### 3.2. Leptin

Consistent with a role for leptin in the regulation of adiposity was the finding by Koyama et al. (1998) that normal lean rats virtually lose all fats when these animals undergo adenovirus gene transfer of the OB gene leading to elevated endogenous leptin production. This treatment, however, is not effective in rats with an electrolytic lesion in the ventrobasal hypothalamus (Koyama et al., 1998), suggesting that this brain region is required for leptin action. Furthermore, relatively low doses of leptin given into the third cerebral ventricle of non-mutant rats (Van Dijk et al., 1996) and mice (Campfield et al., 1995), and even lower doses directly into the ventromedial and arcuate hypothalamic nuclei (Satoh et al., 1997), are much more potent to reduce food intake than when leptin is delivered peripherally. Other evidence pointing in the direction that leptin interacts with the CNS are findings that third cerebral ventricular administration of anorexigenic doses of leptin increases expression of c-Fos (Van Dijk et al., 1996) and other markers of neuronal activity (Elmquist et al., 1998) in several CNS regions including the ventrobasal hypothalamus. Evidence for a CNS action of leptin on lipid metabolism was provided by some of our studies in which normal lean rats underwent chronic third cerebral ventricular leptin treatment over several days (Van Dijk et al., 1999). While producing sustained anorexia, chronic third cerebral ventricular leptin treatment caused rats to lose more fat than a group of control rats receiving a similar amount of food (i.e., pair-fed rats). This appeared to be due to the fact that third cerebral ventricular leptin treatment accentuated lipid oxidation (measured by indirect calorimetry) relative to pair-fed controls. This might underlie the observation that plasma levels of free fatty acids and ketones remained lower than in pair-fed controls. The finding that third cerebral ventricular leptin treatment stimulated thermogenesis suggests that part of the energy arising from increased breakdown of fat and stimulated β-oxidation is dissipated in the form of heat (Van Dijk et al., 1999; Van Dijk, 2001). Consistent with these effects of leptin on metabolism is the finding of Shi et al. (1998) that chronic third cerebral ventricular leptin-treated rats become more insulin-sensitive than pair-fed controls. Essentially the opposite occurs in mice with brain-specific targeted deletion of leptin receptors (Cohen et al., 2001). Among others, these mice have markedly elevated fat accumulation in the liver; a metabolic abberation indicative of insulin resistance (Cohen et al., 2001). The latter phenotype did not occur when leptin signaling, specifically in the liver, was compromised by tissue-specific deletion (Cohen et al., 2001). Consistent with the idea that central leptin signaling is able to regulate hepatic lipid accumulation is the observation by Cohen et al. (2002) that third cerebral ventricular leptin treatment in rats is able to down-regulate hepatic $SCD_1$ transcription.

With respect to the endocrine function of adipose tissue, we have recently begun to investigate whether leptin given into the CNS of rats is able to alter adipose hormone secretion and transcription. Again, rats received chronic third cerebral ventricular leptin infusion, and, while producing anorexia and body weight loss, leptin prevented a fall in the plasma level of adiponectin whereas the plasma adiponectin level decreased by 40% in pair-fed rats. This could

not be attributed to peripheral leptin nor insulin levels, since these levels in third cerebral ventricular leptin-infused rats were indistinguishable from those in pair-fed controls (see Fig. 2).

It is currently unknown how leptin via an action in the CNS is able to regulate adiponectin gene expression or whether it is able to regulate TNF-α and/or resistin synthesis and secretion at all (or that of other adipocyte secretory factors including interleukin 6, vascular endothelial growth factor, or enzymes such as lipoprotein lipase). We did, however, observe recently that chronic third cerebral ventricular infusion of leptin in rats caused a fourfold increase in the mRNA level of PPAR-γ in retroperitoneal fat relative to that in pair-fed controls or ad libitum feeding control rats (de Vries et al., in preparation). Thus, thiazolidinediones and increased CNS leptin signaling might affect overlapping pathways in adipose tissue, involving PPARγ activation and activation of adiponectin synthesis, through which whole-body insulin action is increased and metabolic functions are improved. The CNS outflow pathways controlled by leptin, which are able to affect endocrine activity of adipose tissue, are currently unraveled. Possible mechanisms might include alterations in autonomic nervous system outflow to adipose tissue (Commins et al., 1999; Fliers et al., 2002) and alterations in the activity of several hypothalamo-pituitary axes, including thyroid (Pucci et al., 2000), adrenal (Masuzaki et al., 2001) and growth hormone (Nam and Lobie, 2000), and gonadal (Chehab, 2000) axes. It is important to realize, however, that the abovementioned influences can vary greatly depending on the type of fat deposition (i.e., abdominal, subcutaneous or brown fat).

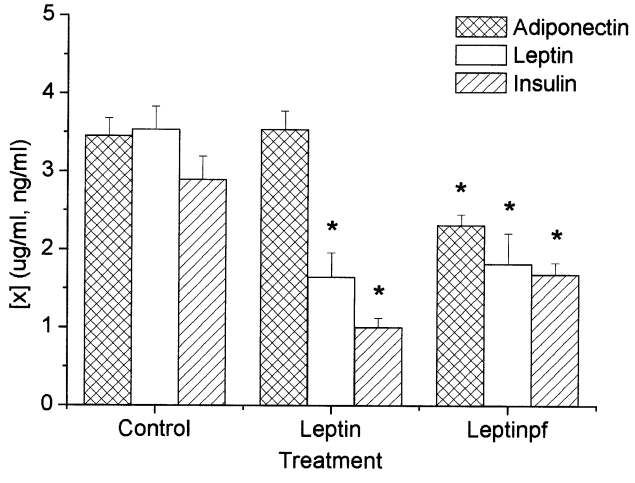


Fig. 2. Effect of 7-day third-cerebral ventricular infusion (by use of osmotic minipumps) of leptin (10 μg/day) on plasma levels of adiponectin (μg/ml), leptin (ng/ml) and insulin (ng/ml) in male Wistar rats. Effects were compared to animals infused into the third cerebral ventricles with saline that were either fed ad libitum (control) or received the same daily amount of food as was daily eaten by the leptin-treated rats (leptinpf). All data are expressed as average change (± S.E.M.). Asterisk indicates a significant difference relative to saline treatment.

## 4. Specific neuronal networks involved in peripheral insulin action and fuel metabolism

As with glucocorticoids, several studies indicate that leptin mediates its effects on food intake, metabolism and fuel partitioning through modulatory actions on neuropeptide producing neuronal cell bodies in the arcuate hypothalamus. Opposed to the stimulatory effects of glucocorticoids on NPY synthesis (Zakrzewska et al., 1999a), leptin reduces the synthesis of NPY as well as its co-synthesized orexigenic neuropeptide, agouti-related protein (AgRP) (Schwartz et al., 2000). Another leptin-receptive cell type located in the arcuate hypothalamus co-synthesizes cocaine-amphetamine related transcript (CART) and pro-opiomelanocortin (POMC) (Schwartz et al., 2000). From the latter, α-melanocyte stimulating hormone (α-MSH) is cleaved. While CART (Kristensen et al., 1998) and α-MSH (Tsujii and Bray, 1989) reduce food intake and promote body fat loss when these neuropeptides are infused into the cerebral ventricles of experimental animals, leptin consistently up-regulates the synthesis of these neuropeptides in fasting and leptin-deficient rodents (Schwartz et al., 2000). The neuronal cell bodies that produce NPY/AgRP and CART/POMC project intra-hypothalamically as well as extra-hypothalamically (Elmquist et al., 1998), and may affect food intake directly through changing the sensation of hunger and/or satiety, or via alterating the activity of downstream neurotransmitter system, including, for example, corticotropin-releasing hormone (Van Dijk, 2001), melanin-concentrating hormone (MCH) (Tritos and Maratos-Flier, 1999), and the neurotrophin-regulated gene product called VGF (nonacronymic) (Hahm et al., 2002).

Although α-MSH is one of the many cleavage products of POMC, it is probably most relevant for regulation of energy balance relative to other POMC products. α-MSH acts agonistically on brain melanocortin receptors (Adan and Gispen, 2000) and competes with AgRP, which is an endogenous antagonist on melanocortin receptors (Ollmann et al., 1997). The melanocortin system received much attention over the last few years since obesity can occur from different mutations in it in humans (Farooqi, 2003; Vaisse et al., 2000). In addition, mice with a targeted deletion in the gene encoding the melanocortin $MC_4$ receptor become morbidly obese as well (Huszar et al., 1997). The melanocortin $MC_4$ receptor is widely expressed in the CNS, including in regions involved in the control of ingestive behavior, sympathetic outflow to peripheral tissues and organs, and neuroendocrine axes (Liu et al., 2003). There are several lines of evidence demonstrating the involvement of the brain melanocortin system in leptin action. The first one came from our rat study in which the anorexigenic effect of third cerebral ventricular injected leptin was prevented by co-administration of the melanocortin $MC_{3/4}$ receptor antagonist, SHU9119, in a dose which was by it self unable to alter food intake (Seeley et al., 1997). Others found that third cerebral ventricular SHU9119 administration was able to block the effects of co-administered

leptin to stimulate sympathetic outflow to peripheral organs (Haynes et al., 1999) and to stimulate brown-adipose tissue thermogenesis (Satoh et al., 1998). We have observed that chronic third cerebral ventricular SHU9119 treatment in rats produces hyperphagia, obesity and hypothermia, despite peripheral hyperleptinemia (Adage et al., 2001). Recently, we found that the opposite occurs when an agonist of the melanocortin $MC_4$ receptor is given chronically into the third cerebral ventricle of rats over several days. In addition, we observed that, analogous to the effects of central leptin infusion, chronic infusion of the melanocortin $MC_4$ receptor agonist into the third cerebral ventricle caused an increase in the circulating level of adiponectin. Consistent with this is the finding of the group of Rosetti (Obici et al., 2001) that increased activity of brain melanocortin receptors by third cerebral ventricular α-MSH infusion augments insulin-induced glucose uptake in peripheral tissue, whereas third cerebral ventricular administration of SHU9119 causes the opposite effect (Obici et al., 2001). The mechanisms by which increased CNS leptin signaling, presumably via increased brain melanocortin receptor activity, increases insulin action and exerts other mentioned metabolic effects are as yet unknown. If any, it appears not to rely on the integrity of the hypothalamo-pituitary adrenal axis since chronic alterations in brain melanocortin receptor activity are not associated with changes in glucocorticoid levels (Huszar et al., 1997), nor does adrenal insufficiency prevent obesity associated with low brain MC receptor activity (Yaswen et al., 1999). In contrast, hyperactivity of the NPY system in rats is known to cause hyperphagia, obesity, and insulin resistance, as well as hypercorticoidism (Sainsbury et al., 1997). In this case, the obesity as well as insulin resistance were attenuated when NPY is given into the third cerebral ventricle rats which had their adrenals removed (Sainsbury et al., 1997). Finally, also hyperactivity of the MCH systems leads to obesity and insulin resistance (Ludwig et al., 2001), but the mechanism is unclear. MCH neurons, however, have very widespread connection in the CNS, including the autonomic preganglionics (Elias et al., 1998), and could therefore influence peripheral fuel fluxes through altered autonomic tone. Taken together, pivotal CNS neuronal networks are involved in leptin signaling, and reduce ingestion as well modulate autonomic and neuroendocrine outflow to facilitate lipid breakdown and increase peripheral insulin sensitivity. Disturbances in these mechanisms can lead to different forms of obesity and associated metabolic syndromes including insulin resistance.

## 5. Conclusion and remarks

It has been mentioned above that leptin can, via an action in the CNS, alter endocrine activity of adipose tissue leading to increased adiponectin release, which in turn increases peripheral lipid oxidation and insulin sensitivity. Given the surplus of high-palatable foods in our western-industrialized societies, however, the homeostatic feedback systems that regulate energy balance can propably easily be overridden and lead to increases in fat mass. Since increased fat mass, and in particular that of the abdominal compartment, exposes the liver to elevated levels of free fatty acids, this could amplify HPA axis activity leading to elevated levels of glucocorticoids. In turn, glucocorticoids can contribute to reduced insulin action in peripheral tissues and organs, or by directly down-regulating adiponectin release. Within the CNS, glucocorticoids can reduce leptin action by regulating the activity of CNS neuropeptide involved in food intake, neuroendocrine outflow and metabolism, generally in the opposite direction (e.g., Zakrzewska et al., 1999a), or potentially by down-regulating the intraneuronal signaling cascade for leptin (Madiehe et al., 2001; Makimura et al., 2000). Together, these effects might be a major cause of leptin resistance (Zakrzewska et al., 1997), and thus ultimately have an impact on endocrine activity of adipose tissue, peripheral metabolic pathways, and whole body insulin sensitivity. Thus, according to this scheme, feeding a high-fat diet puts subjects at risk for developing obesity and related metabolic diseases. Psychological stress leading to chronically elevated levels of glucocorticoids is probably another, or additional, cause of obesity and related metabolic diseases (Ur et al., 1996; Bjorntorp, 2001). At this point, the biological relevance of this mechanism may not be clear. However, recent studies from our laboratory have shown that rats fed a high-fat diet are less affected by psychosocial stress in terms of fever development, disturbances in locomotor activity and feeding behavior (Buwalda et al., 2001) than rats fed a high-carbohydrate diet. At a later stage, however, the high-fat-feeding rats, but not the carbohydrate-feeding rats, develop insulin resistance (Van Dijk and Buwalda, 2003). It may therefore be suggested that the increased risk of obesity and related metabolic diseases as a cause of stress and high-fat feeding is simply a trade-off, which may not necessarily have hampered the evolutionary success of humans as well as other species.

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